

high affinity binding to IL-15. Whereas IL-2R $\alpha$  is primarily expressed on activated T cells, IL-15R $\alpha$  mRNA has been identified in various tissues and cells. Like IL-2 the IL-15R $\alpha\beta\gamma$  complex signals through JAK1/3 and STAT3/5 pathways (3,28). IL-15 has already been described to be essential for the proliferation and maintenance of CD8<sup>+</sup> memory T cells and acts, at high dose, as a pan T cell and mast cell growth factor (2,26,27). Linder, J. Invest. Dermatol., 110: 457-458 (1998) discloses that IL-15 inhibits apoptosis of keratinocytes in hair bulbs.

However, effective means for promoting hair growth and for treating, preventing and/or ameliorating the diseases referred to hereinabove are still not obtainable but nevertheless highly desirable.

Thus, the technical problem underlying the present invention must be seen as the provision of means for effectively promoting hair growth and for treating, preventing and/or ameliorating hair loss caused by or accompanied with diseases. The technical problem is solved by the embodiments characterized in the claims.

Accordingly, the present invention relates to the use of

- (i) a polynucleotide comprising
  - (a) a nucleic acid sequence as shown in SEQ ID NO: 1 or 3,
  - (b) a nucleic acid sequence encoding an amino acid sequence as shown in SEQ ID NO: 2 or 4,
  - (c) a nucleic acid sequence encoding an amino acid sequence as shown in SEQ ID NO: 2 or 4 having a modified signal peptide, a modified N-terminus and/or a modified C-terminus, or
  - (d) a nucleic acid sequence which hybridises under stringent conditions to any one of (a) to (c);
- (ii) a polypeptide encoded by the nucleic acid as defined in any one of (a) to (d); or
- (iii) a compound which binds to an antibody which specifically recognizes the polypeptide defined in (ii) or which specifically binds to an IL-15 receptor alpha chain

for the preparation of a composition for stimulating hair growth.

The term "polynucleotide" relates to polynucleotides which encode a polypeptide having a biological or antigenic activity of interleukin 15 (IL-15). The structure of various IL-15 polypeptides has been described in the art and representative IL-15 polypeptides are shown in SEQ ID NO: 2 (human IL-15, Accession No. BC018149; gi34783292) and SEQ ID NO: 4

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(mouse IL-15, Accession No. BC023698; gi23271448). Several biological functions of IL-15 are also reported in the art and have been discussed herein before. An essential biological activity of IL-15 is its capability to specifically bind to the IL-15 receptor alpha chain as deposited under (Accession No. BC022705 for mouse IL-15 R $\alpha$  and Accession No. AY316538 for human IL-15 R $\alpha$  (2, Lodolce, Immunity 1998, 9: 669 – 676)). Other well characterized biological activities include its capability to stimulate NK-/NKT cells and memory T-cells (Flamand, J. Clin. Invest, 1996, 97: 1373 - 81; Kv, Science 2000, 288: 675 - 678) and its proliferative effect on lymphoid or mesenchymal cells as well as the prevention of apoptosis after induction with apoptic substances (14). Preferably, said biological activity is the stimulation of hair growth and keratinocytes as demonstrated in the accompanied Examples. An essential antigenic activity is its capability to be specifically recognized by a specific, i.e. non-cross-reactive, IL-15 antibody as disclosed in Shinozaki, J. Clin. Invest, 2002, 109: 951 – 960. Such an IL-15 antibody can also be obtained by routine methods. Preferably, the antibody is a monoclonal antibody. These activities can be tested by routine methods well known in the art and described in the above cited references in detail. Most preferably, the polynucleotides of the present invention have a nucleic acid sequence as shown in SEQ ID NO: 1 (human IL-15) or SEQ ID NO: 3 (mouse IL-15).

Preferably, the IL-15 polynucleotides also encompass variant polynucleotides which are capable to hybridise with the polynucleotides shown in SEQ ID NO: 1 or SEQ ID NO: 3 under stringent hybridisation conditions. More preferably, said conditions are disclosed in Ausubel, 2001, Current protocols in molecular biology. Said polynucleotides are most preferably at least 70 %, at least 80 %, at least 85 %, at least 90 %, at least 95 %, at least 96 %, at least 97 %, at least 98 % or at least 99 % identical with SEQ ID NO: 1 or SEQ ID NO: 3.

The variant polynucleotides of the invention may comprise a modified signal peptide or leader sequence, i.e. amino acids 1 to 48 of SEQ ID NO: 2, amino acids 1 to 48 of SEQ ID NO: 4 and amino acids corresponding thereto in polypeptide variants thereof. Modifications meant hereby are those which increase the secretion of IL-15 from a cell. Biological assays for testing whether a modification increases said secretion are well known in the art and are described in (5) and (6). Most preferably, the signal peptide is modified by replacing it with the signal peptide of CD33 polypeptide (Accession No. NM 02 1293). Moreover, the N- or C-terminal amino acids of the mature polypeptide shown in SEQ ID NO: 2 or SEQ ID NO: 4 or amino acids corresponding thereto in the polypeptide variants may be modified as to increase stability of the mature polypeptides. The stability of mature IL-15 polypeptides can be tested

**Amended set of claims**

1. Use of
  - (i) a polynucleotide comprising
    - (a) a nucleic acid sequence as shown in SEQ ID NO: 1 or 3,
    - (b) a nucleic acid sequence encoding an amino acid sequence as shown in SEQ ID NO: 2 or 4,
    - (c) a nucleic acid sequence encoding an amino acid sequence as shown in SEQ ID NO: 2 or 4 having a modified signal peptide, a modified N-terminus and/or a modified C-terminus, or
    - (d) a nucleic acid sequence which hybridises under stringent conditions to any one of (a) to (c);
  - (ii) a polypeptide encoded by the nucleic acid as defined in any one of (a) to (c); or
  - (iii) a compound which binds to an antibody which specifically recognizes the polypeptide defined in (ii) or which specifically binds to an IL-15 receptor alpha chainfor the preparation of a composition for stimulating hair growth.
2. Use of a polynucleotide, polypeptide or compound as defined in claim 1 for the preparation of a composition for treating, preventing and/or ameliorating hair loss.
3. The use of claim 1 or 2, wherein said composition further comprises a second hair growth stimulating agent.
4. The use of claim 3, wherein said second hair growth stimulating agent is selected from the group consisting of zinc salts of carboxylic acids, saponins, triterpenes, preferably oleanolic acid or ursolic acid, crataegolic acid, celastrol, Asiatic acid, inhibitors of 5-[alpha]-reductase, preferably progesterone, 1,4-methyl-4-azasteroids, preferably 17-[beta]-N,N-diethylcarbamoyl-4-methyl-4-aza-5-[alpha]-androstan-3-one, androgen receptor antagonists, preferably cyproterone acetate, Minoxidil(R), azaelaic acid and derivatives thereof, cyclosporin, triiodothyronine, diazoxide, potassium channel openers, preferably cromakalin, phenytoin, and mixtures thereof, and derivatives of oestrogen, preferably oestradiolvalerate.

5. The use of any one of claims 1 to 4, wherein said composition further comprises a pharmaceutically or cosmetically acceptable carrier.
6. The use of any one of claims 1 to 5, wherein said composition is a pharmaceutical composition.
7. The use of any one of claims 1 to 5, wherein said composition is a cosmetic composition.
8. The use of any one of claims 1 to 7, wherein said composition is formulated as a hair tonic, a hair restorer composition, a shampoo, a powder, a jelly, a hair rinse, an ointment, a hair lotion, a paste, a hair cream, a hair spray and/or a hair aerosol.
9. The use of any one of claims 1 to 8, wherein said composition is to be administered topically to the skin or scalp of a subject.
10. The use of claim 9, wherein said subject is a mammal.
11. The use of claim 10, wherein said mammal is a human, a dog, a cat, a horse, a rabbit, a sheep, a camel, a mouse, a rat, an alpaca, a vicuna, a guanaco or a lama.
12. The use of any one of claims 9 to 11, wherein said subject suffers from genetically determined and/or acquired form of hair loss.
13. The use of claim 12, wherein said genetically determined or acquired form of hair loss is alopecia areata, alopecia subtotalis, alopecia totalis, trichotillomania or drug induced alopecia.
14. A transgenic non-human animal comprising a nucleic acid as defined in claim 1, wherein said nucleic acid is specifically expressed in the keratinocytes of the hair bulb, in the Langerhans cells, in the melanocytes, in the dendritic epidermal T-cells, in the mast cells, in cutaneous nerve fibres or in fibroblasts.
15. A method for stimulating hair growth in a non-human animal comprising the steps of:

- (a) Transforming said animal with a nucleic acid as defined in claim 1; and
  - (b) Expressing the polypeptide encoded by said nucleic acid.
16. A method for manufacturing non-human animal hair comprising the steps of:
- (a) Transforming said non-human animal with a nucleic acid as defined in claim 1; and
  - (b) Expressing the polypeptide encoded by said nucleic acid.
17. The method of claim 15 or 16, wherein said IL-15 polypeptide is expressed under the control of a regulatory element.
18. The method of claim 17, wherein said regulatory element enables specific expression in the keratinocytes of the hair bulb, in the Langerhans cells, in the melanocytes, in the dendritic epidermal T-cells, in the mast cells, in cutaneous nerve fibres or in fibroblasts.
19. The method of any one of claims 16 to 18, further comprising the step of administering to the skin and/or scalp of a non-human animal the composition as defined in claim 1.
20. A method for manufacturing non-human animal hair comprising the step of administering to the skin and/or scalp of a non-human animal the composition as defined in claim 1.
21. The method of any one of claims 16 to 20, further comprising the step of obtaining the hair of said non-human animal.
22. The transgenic non-human animal of claim 14 or the method of any one of claims 15 to 21, wherein said animal is a dog, a cat, a horse, a rabbit, a sheep, a camel, a mouse, a rat, an alpaca, a vicuna, a guanaco or a lama.
23. A method of treating, preventing and/or ameliorating a subject which suffers from hair loss comprising the step of administering a composition as defined in claim 1 in an effective dosage to said subject.

5/5

Figure 5

## mIL-15 mRNA - modified Sequence

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1  cttctgtcca gccactcttc cccagagttc tcttcttcat cctccccctt gcagagtagg
61  gcagcttgca ggtcctcctg caagtctctc ccaattctct gcgccccaaa gacttgcagt
121 gcatctcctt acgcgctgca gggaccttgc cagggcagga ctgcccccg cagttgcag
181 agttggacga agacgggatc ctgctgtgtt tggaaggctg agttccacat ctaacagctc
241 agagaggtca ggaaagaatc caccttgaca catggccctc tggtctttca aagcactgcc
301 tcttcatggt ccttgctggt gaggtcctta agaacacaga aacctatgtc agcagataac
361 cagcctacag gaggccaaga agagttctgg atggatggca gctggaagcc catcgccata
421 gccagctcat cttcaacatt gaagctctta cctgggcatt aagtaatgaa aattttgaaa
481 ccataatga ggaatacatc catctcgtgc tacttgtgtt tccttctaaa cagtcacttt
541 ttaactgagg ctggcattca tgtcttcatt ttgggctgtg tcagtgtagg tctccctaaa
601 acagaggcca actggataga tgtaagatat gacctggaga aaattgaaag ctttattcaa
661 tctattcata ttgacaccac tttatacact gacagtgact ttcatcccag ttgcaaagtt
721 actgcaatga actgctttct cctggaattg caggttattt tacatgagta cagtaacatg
781 actcttaatg aaacagtaag aaacgtgctc taccttgcaa acagcactct gtcttctaac
841 aagaatgtag cagaatctgg ctgcaaggaa tgtgaggagc tggaggagaa aaccttcaca
901 gagtttttgc aaagctttat acgcattgtc caaatgttca tcaacacgtc ctgactgcat
961 gcgagcctct tccgtgttct tgttattaag gtacctccac ctgctgctca gaggcagcac
1021 agctccatgc atttgaaatc tgctgggcaa actaagcttc ctaacaagga gataatgagc
1081 cacttgatc acatgaaatc ttggaaatga agagaggaaa agagctcgtc tcagacttat
1141 ttttgcttgc ttatttttaa tttattgctt catttgtaac tatttgtaat ataacagaag
1201 atgtggaata aagttgtatg gatattttat caattgaaat taaaaaaaaa

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